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Enantioselective binding of dipeptides using acyclic receptors†

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Novel receptors featuring a 2,6-diamidopyridine 'head' group and bearing sulfonamidopeptide sidearms have been prepared on the solid-phase; one receptor showed high selectivity for *N*-Cbz-D-Ala-D-AlaOH over its enantiomer *N*-Cbz-L-Ala-L-AlaOH, but absolute binding constants were relatively weak, which can be understood in terms of receptors which have to unfold, breaking intramolecular hydrogen bonds, in order to accommodate the guests.

While several macrocyclic hosts have been prepared and shown to bind amino acids or peptide fragments with excellent selectivity,[1] during the last decade a different class of receptors for peptides called 'tweezer receptors' or 'two-armed' receptors have been developed.[2,3] Many of these systems bind substrates, and particularly peptides, with selectivities which are in many ways surprising, given the apparent flexibility of such acyclic receptor structures.

Whereas in many such 'two-armed' receptor systems the head group plays only a limited role in the binding of the guest, the group in Southampton have recently developed receptors utilising a diamidopyridine as the head group which can specifically bind to carboxylic acid functionality, and such receptors, with peptidic arms, have proven to be selective receptors for peptides with a carboxylic acid terminus.[3] In this paper we report studies on novel 'two-armed' receptors consisting of a 2,6-diamidopyridine head group bearing sulfonamidopeptide sidearms,[4] one of which, in particular, shows high binding selectivity for the dipeptide *N*-Cbz-D-Ala-D-AlaOH (Cbz = benzyloxycarbonyl) over its enantiomer *N*-Cbz-L-Ala-L-AlaOH. In contrast to the CONH moiety of peptides, which provides both a strong hydrogen-bond donor and acceptor, the SO_2NH moiety of sulfonamidopeptides provides a very strong donor NH, but the SO_2 group is only a weak acceptor.[5,6]

The synthesis of the novel receptors was accomplished on the solid phase starting from *N*-Fmoc-Gly Wang resin **1** (loading 0.88 mmol g^{-1}) (Scheme 1). After deprotection of the amino-group, diamidopyridine derivative **2**[3] was coupled using a 20% excess of resin, and the unreacted amino groups on the resin were capped with acetylimidazole. Deprotection of the Fmoc groups and coupling with the L-valine-derived β-*N*-Fmoc-amino sulfonyl chloride using DMAP as catalyst and dimethylketene methyl trimethylsilyl acetal (MTDA) as HCl scavenger,[5,7] gave a resin bound disulfonamide. Cleavage of Fmoc protection, followed by acetylation gave the desired disulfonamide derivative, which was cleaved from the resin by a direct basic methanolysis or by acidic TFA–H_2O cleavage followed by esterification to give receptor **4**. Alternatively the resin bound sulfonamide could be deprotected and subjected to a further coupling with the L-valine-derived β-*N*-Fmoc-amino sulfonyl chloride, followed by deprotection, acetylation and cleavage to give receptor **5**.

Binding studies with receptors **4** and **5** were carried out with a series of substrates in deuteriochloroform, using a standard NMR titration experiment.[8] Titration of **4** with simple amino acid derivatives such as *N*-Cbz-L-AlaOH or *N*-Boc-L-AlaOH gave modest binding constants (K_{ass} = 207 and 119 M^{-1} respectively) and little apparent enantioselectivity (*N*-Cbz-L-AlaOH: K_{ass} = 207 M^{-1}, *N*-Cbz-D-AlaOH: K_{ass} = 270 M^{-1}, Table 1). Binding of all three amino acid substrates led to significant downfield shifts of the amidopyridine NH^1 (≥0.5 ppm).[9] Binding of dipeptide guests gave slightly higher binding constants, and titration of **4** with, for example, *N*-Cbz-L-

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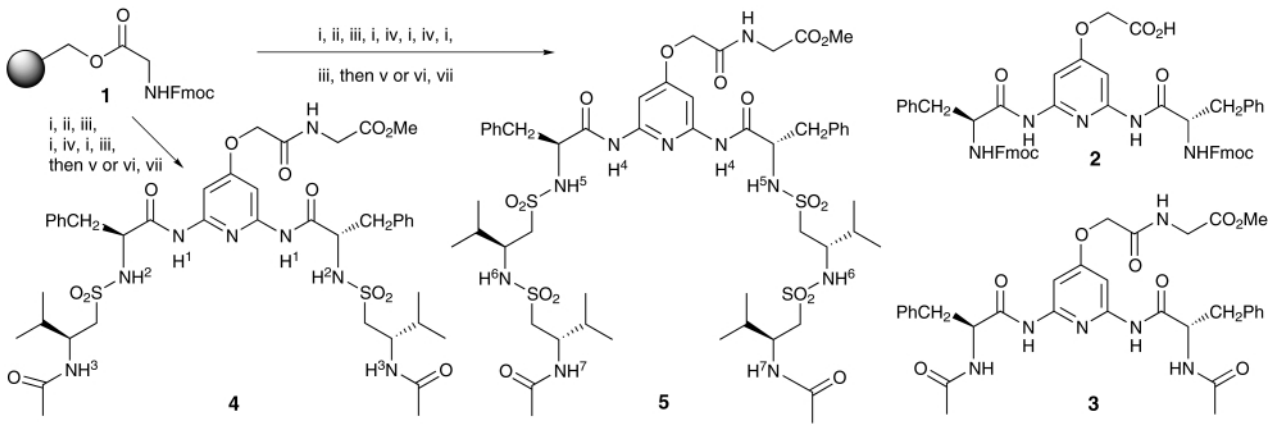

Scheme 1 *Reagents and conditions*: (i) piperidine (20% in DMF); (ii) **2**, DIC, HOBT, DMAP, DCM; (iii) AcIm, DCM; (iv) $FmocNHCH(CHMe_2)CH_2SO_2Cl$, DMAP, MTDA, DCM, two cycles; (v) Et_3N, MeOH, DMF; (vi) TFA, H_2O; (vii) EDC, DMAP, MeOH, THF.

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Ala-L-AlaOH gave a binding constant of 245 M^{-1} and led to downfield shifts of both the amidopyridine and sulfonamide NH's (NH^1: $\Delta\delta$ = 0.25 ppm, NH^2: $\Delta\delta$ = 0.30 ppm) and an upfield shift of acetamide NH^3 ($\Delta\delta$ = 0.12 ppm). Binding of dipeptides was also associated with downfield shifts for both the amide and carbamate NH signals of the guest ($\Delta\delta$ = 0.10–0.35 ppm). Titration with *N*-Cbz-D-Ala-D-AlaOH led to little change to the amidopyridine NH^1 ($\Delta\delta \leqslant 0.03$ ppm), but analysis of the downfield shift of sulfonamide NH^2 ($\Delta\delta$ = 0.28 ppm) and the upfield shift of acetamide NH^3 ($\Delta\delta$ = 0.19 ppm) gave a binding constant of 242 M^{-1} indicating that **4** also shows little enantioselectivity for dipeptides (K_{ass} for *N*-Cbz-L-Ala-L-AlaOH = 245 M^{-1}, Table 1). Thus receptor **4** does appear to bind dipeptides and simple amino acid derivatives, but the absolute binding constants are rather low in comparison to other diamidopyridine based receptors, and the shifts of the various NH signals on binding are also rather small in comparison to those seen in other related receptor systems.[1b,10,11] We therefore studied receptor **5**, reasoning that with additional binding functionality in the longer sidearms it might show increased affinity for peptide guests. In practice, receptor **5** initially gave absolute binding constants somewhat lower than **4** (Table 1) and again only small shifts were observed for the various NH signals on addition of guest. However, binding of the *N*-Cbz-Ala-AlaOH dipeptide enantiomers showed marked selectivity. Titration of **5** with *N*-Cbz-L-Ala-L-AlaOH gave a binding constant of 107 M^{-1} and led to a downfield shift of 0.3 ppm for the amidopyridine NH^4 but only small changes to the sulfonamide and acetamide NH's (NH^5, NH^6, NH^7, $\Delta\delta \leqslant 0.07$ ppm). In contrast, titration of **5** with *N*-Cbz-D-Ala-D-AlaOH gave a binding constant of 2404 M^{-1} with a particularly large downfield shift for the sulfonamide NH^6 ($\Delta\delta$ = 0.72 ppm) but an upfield shift for sulfonamide NH^5 ($\Delta\delta$ = 0.38 ppm) and even a small upfield shift for the amidopyridine NH^4 ($\Delta\delta$ = 0.05 ppm)! Binding of *N*-Cbz-D-Ala-D-AlaOH was also accompanied by significant shifts of several CH signals of the host (up to 0.37 ppm) and significant shifts for the guest NH protons ($\Delta\delta \approx 0.5$ ppm).[9]

The small shifts on binding for the NH signals, and generally low absolute binding constants, for receptors **4** and **5**, can be rationalised by considering the unbound conformation of the receptors, which were investigated in detail by NMR.

In the ^{1}H-NMR spectra of **4** and **5** in $CDCl_3$ peaks are rather broad, indicating slow conformational equilibria at room temperature (on the NMR time scale). The NH protons, for both **4** and **5** display a very small concentration dependence in $CDCl_3$ indicating that the receptors do not aggregate in the concentration range considered (0.5–20 mM). At a concentration of 8.5 mM in $CDCl_3$ the signals of the amidopyridine NH protons appear at unexpectedly low field [$NH^1$1(**4**): 9.41, NH^4(**5**): 9.32 ppm compared to standard values[1b,11] 8.0–8.5 ppm], and the sulfonamide NH protons are also strongly deshielded [NH^2(**4**): 6.32, NH^5(**5**): 5.78, NH^6(**5**): 6.32 ppm compared to their standard values[5] 4.50–4.74 ppm], while the acetamide protons, NH^3(**4**) and NH^7(**5**), are closer to their normal chemical shifts (6.2 compared to 6.0–6.2 ppm). In comparison, the spectrum of the control compound **3** (Scheme 1) in $CDCl_3$ is perfectly resolved, and the amidopyridine NH signal is found at 8.83 ppm (for a concentration of 8.5 mM), whereas the acetamide NH proton is found at 6.56 ppm. This suggests that receptors **4** and **5** collapse to give folded structures stabilised by intramolecular hydrogen bonds. Such a conclusion is supported by NOESY and ROESY experiments, which revealed a large number of NOE contacts consistent with a folded structure.

Thus binding of substrates by **4** or **5** involves a degree of unfolding of the receptor, and breaking of intramolecular hydrogen bonds (with an associated energetic cost), to allow interaction with the guest, resulting in rather low binding constants and only small overall changes to the signals for the hydrogen bonding NH protons. In particular, the only significant change in the NMR for **5**, on binding of *N*-Cbz-L-Ala-L-AlaOH, is a downfield shift of the amidopyridyl NH^4, indicating breaking of an intramolecular hydrogen bond to this NH and binding with the carboxylic acid of the guest. In contrast, the binding of *N*-Cbz-D-Ala-D-AlaOH by **5** leads to significant shifts of several CH as well as NH signals throughout the spectrum (although there is little overall shift to the amidopyridine NH^4) suggesting a much more dramatic conformational change for the receptor on binding this guest[12] and that binding of this guest is sufficiently strong to overcome the penalty of unfolding the receptor. In any event such high enantioselectivity, > 20 : 1 (effectively discriminating between methyl groups and hydrogen atoms), has rarely been observed in synthetic receptors,[1,10] and is particularly noteworthy in such a structurally simple acyclic receptor, which appears to lack much, if any, preorganisation for binding.

Table 1 Binding constants[a] (K_{ass}) for the 1 : 1 complexes[b] formed between receptors **4** and **5** and various amino acid and dipeptide derivatives, in $CDCl_3$ at 25 °C

	K_{ass}[a]/M^{-1}	
Substrate	**4**	**5**
N-Cbz-L-AlaOH	207	32
N-Boc-L-AlaOH	119	—[c]
N-Cbz-D-AlaOH	270	—[c]
N-Cbz-L-Ala-L-AlaOH	245	107
N-Boc-L-Ala-L-AlaOH	361	—[c]
N-Cbz-D-Ala-D-AlaOH	242	2404[d]

[a] Calculated from the chemical shifts of various ^{1}H signals of **4** and **5**. Unless otherwise stated errors for K_{ass} estimated as < 10%.[8] [b] 1 : 1 stoichiometry was confirmed by Scatchard analysis.[13] [c] Experiment not performed. [d] Error was estimated as < 5%, using data from five different ^{1}H signals.

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